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Review

Paediatric Strategy Forum for medicinal product development of DNA damage response pathway inhibitors in children and adolescents with cancer: ACCELERATE in collaboration with the European Medicines Agency with participation of the Food and Drug Administration

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Abstract DNA damage response inhibitors have a potentially important therapeutic role in paediatric cancers; however, their optimal use, including patient selection and combination strategy, remains unknown. Moreover, there is an imbalance between the number of drugs with diverse mechanisms of action and the limited number of paediatric patients available to be enrolled in early-phase trials, so prioritisation and a strategy are essential. While PARP inhibitors targeting homologous recombination-deficient tumours have been used primarily in the treatment of adult cancers with *BRCA1/2* mutations, *BRCA1/2* mutations occur infrequently in childhood tumours, and therefore, a specific response hypothesis is required. Combinations with targeted radiotherapy, ATR inhibitors, or antibody drug conjugates with DNA topoisomerase I inhibitor-related warheads warrant evaluation. Additional monotherapy trials of PARP inhibitors with the same mechanism of action are not recommended. PARP1-specific inhibitors and PARP inhibitors with very good central nervous system penetration also deserve evaluation. ATR, ATM, DNA-PK, CHK1, WEE1, DNA polymerase theta and PKMYT1 inhibitors are early in paediatric development. There should be an overall coordinated strategy for their development. Therefore, an academia/industry consensus of the relevant biomarkers will be established and a focused meeting on ATR inhibitors (as proof of principle) held. CHK1 inhibitors have demonstrated activity in desmoplastic small round cell tumours and have a potential role in the treatment of other paediatric malignancies, such as neuroblastoma and Ewing sarcoma. Access to CHK1 inhibitors for paediatric clinical trials is a high priority. The three key elements in evaluating these inhibitors in children are (1) innovative trial design (design driven by a clear hypothesis with the intent to further investigate responders and non-responders with detailed retrospective molecular analyses to generate a revised or new hypothesis); (2) biomarker selection and (3) rational combination therapy, which is limited by overlapping toxicity. To maximally benefit children with cancer, investigators should work collaboratively to learn the lessons from the past and apply them to future studies. Plans should be based on the relevant biology, with a focus on simultaneous and parallel research in preclinical and clinical settings, and an overall integrated and collaborative strategy.

1. Introduction

The DNA damage response (DDR) is a complex process that includes signal transduction pathways required for preserving genomic stability. Dysregulation of the DDR can cause several human disorders, including cancer, accelerated ageing and developmental abnormalities. There has been rapid growth in the development of DDR pathway inhibitors, including agents targeting PARP, ATR, ATM, DNA-PK, PKMYT1, DNA polymerase theta (Polθ, also referred to as POLQ) and WEE1. The optimal use of these drugs, including patient selection and combination treatment strategies, remains largely unknown for paediatric cancers. Moreover, there is an imbalance between the high number of DDR targeting drugs, with diverse mechanisms of action, and the limited number of paediatric patients available to be enrolled in early-phase trials; thus, prioritisation and a harmonised strategy to study these agents through collaboration are essential.

To date, several PARP inhibitors are approved in adult cancer patients with *BRCA* mutations or DDR biomarker-positive malignancy, and the investigation of this class of products has been ongoing in several paediatric cancer trials since 2009 [1]. There are both ongoing paediatric clinical trials evaluating these agents and active regulatory submissions of initial Paediatric Study Plans (iPSPs) and Paediatric Investigational Plans (PIPs). Paediatric patients enrolled in studies of PARP inhibitors have significant variability in target histological tumour types, and tumour-agnostic approaches are being explored. Fewer studies have evaluated other DDR targeting agents, including ATR, ATM, DNA-PK, CHK1, WEE1, Polθ and PKMYT1 inhibitors in paediatric patients.

The multistakeholder Paediatric Strategy Forum organised by ACCELERATE [2,3] in collaboration with the European Medicines Agency (EMA) with the participation of the US Food and Drug Administration (FDA) aimed to evaluate the science, facilitate dialogue, share information and foster prioritisation [4–11]. The Forum addressed key issues in the ongoing development of PARP and other DDR pathway inhibitors, specifically: (1) How best to use biomarkers to identify the optimal patient populations for these medicinal products? (2) Which genomic aberrations render tumours sensitive to these agents? (3) The most effective trial designs to evaluate these agents. (4) Based on knowledge of biology, what are the most promising rational combinations, including novel-novel combinations?

The meeting was held at the EMA, Amsterdam, on 27 and 28 October 2022. There were 124 participants, 63 in person and 61 virtually: 41 international clinical paediatric oncology and biology experts from Europe, the United States of America (USA), Canada and Japan; an expert in adult anti-cancer drug development: 43 representatives from six pharmaceutical companies in Europe, Canada and the USA (AstraZeneca, GSK, Merck KGaA, Pfizer, Repare Therapeutics, Roche); seven patient advocates from Europe, the USA and Nigeria (representatives from Andrew McDonough B+ Foundation, Children's Cancer Cause, The Dorcas Cancer Foundation, Imagine for Margo, KickCancer, Solving Kids' Cancer, Zoé4life and Childhood Cancer International Europe); 30 regulators from the EMA (including the Paediatric Committee [PDCO]) and national competent authorities within the EU regulatory network, European Health Technology Agencies and US FDA as observers; and two organisers. An overview of the biology of the DDR pathway in childhood cancer, experience with PARP inhibitors in adults and children and discussion of the relevant biomarkers was first presented by academic experts to form a basis for discussion of other DDR pathway inhibitors. Details of 15 inhibitors of the DDR pathway were presented by companies or academic investigators. The Forum concluded with the patient advocates' perspective and a multistakeholder strategic discussion.

2. Biology of the DDR pathway and rationale for DDR pathway inhibitors in paediatric cancer

Human cells continually encounter DNA damage from endogenous and exogenous sources with DNA single-strand breaks (SSBs) being the most common while DNA double-strand breaks (DSBs) being the most detrimental. In order to repair such DNA damage, cells leverage the coordinated and complex DDR signalling cascade. This begins with an initial wave of DNA damage recognition, for example, PARP has a DNA-binding domain which recognises SSB and DSB, ATR binds to exposed single-stranded DNA coated by replication protein A, whereas ATM and DNA-PK are recruited by MRN and Ku, respectively, to the sites of DSB and mediate distinct repair pathways. This initial DNA recognition is then followed by a signalling cascade, including the activation of the mediator proteins involved in cell cycle regulation and recruitment of additional repair proteins (e.g. ATR and ATM activate CHK1 and CHK2, respectively; DNA-PK recruits the endonuclease Artemis). This signalling cascade thus involves recognition and correction of DNA damage, as well as inhibition of cell cycle progression, thus providing time for repair to occur [12–16] (Figs. 1 and 2).

There are different processes for DSB repair: (1) classical non-homologous end joining (c-NHEJ); (2) homologous recombination (HR); (3) single-strand annealing (SSA) and (4) alternative end joining (a-EJ). Some proteins are involved in multiple repair processes and others play a preferentially focused role in the DDR pathway (e.g. DNA-PK with c-NHEJ as well as POLQ with a-EJ) [14,17,18]. PARP1 and PARP2 enzymes participate in the response of SSB in DNA [12] but are also involved in DNA damage signalling. Compared to

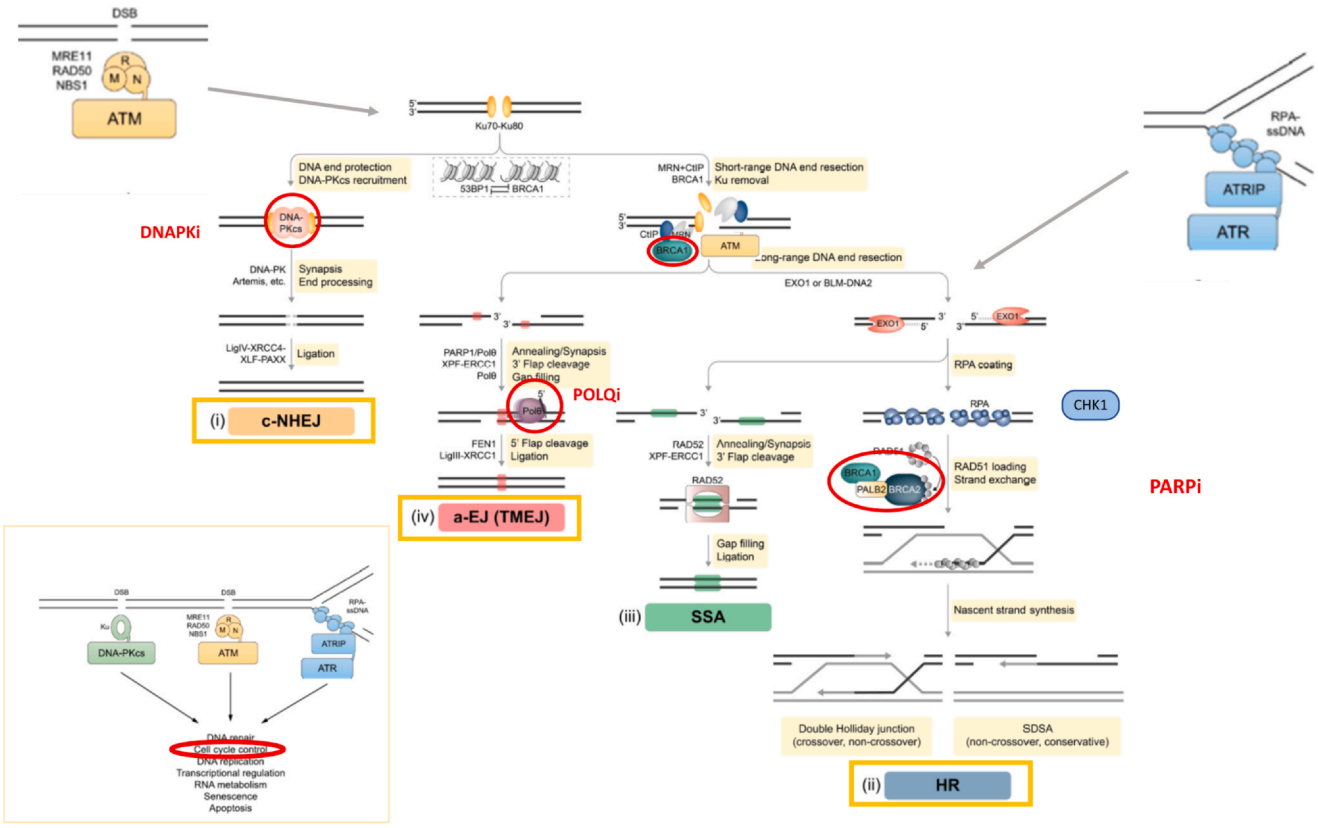

Fig. 1. DNA damage response pathway. a-EJ, alternative end joining; c-NHEJ, classical non-homologous end joining; HR, homologous recombination; SSA, single-strand annealing.

healthy cells, cancer cells have a higher degree of DNA damage due to oncogene-induced replication stress, accumulation of R-loops (RNA/DNA hybrids, which form when transcription and replication collide), aberrant DNA repair processes (e.g. PGBD5 [19], alternative lengthening of telomeres [ALT]), as well as acquired defects through loss/inactivation of aspects of the DDR machinery. As such, cancer cells therefore rely on an enhanced DDR for their survival [15,16,20,21].

Based on the primarily activated kinase, the DDR response includes overlapping pathways, which are potentially targetable at different levels: (1) ATM-CHK2-CDC25A-CDK2 and p53-p21 preferentially respond to DSB to inhibit cell cycle progression into S-phase; (2) ATR-CHK1-CDC25C-CDK1 preferentially respond to replication-associated damage in S and G2/M to inhibit cell cycle progression into mitosis; (3) WEE1 and the related PKMYT1 are involved in the DDR response downstream at the level of CDK1 and CDK2, that is, at the level of regulation of cell cycle checkpoints; and (4) DNA-PK preferentially responds to DSB when HR is unavailable [15,17,18,22].

Stress on the genome (DNA damage, errors during DNA replication and aberrant DNA replication signalling or collision with the transcription machinery) results in replication stress/stalled replication fork during DNA replication and thereby single-stranded DNA being exposed. ATR and its downstream target CHK1 are recruited to alleviate replication stress [23]. If effective in their repair, replication stress is resolved and DNA synthesis resumes. However, if these regulatory proteins fail to stabilise the stalled replication fork, it can collapse and DSB occurs unless alternative pathways (such as those mediated by the complementary ATM/CHK2 pathway) repair the break. Replication stress arises from events such as *RB1* loss of function, *CCNE1* amplification, *MYC*/*MYCN* amplification, oncogenic fusions and trapped PARP inhibitors.

Synthetic lethality (whereby inhibiting two targets together results in cell death but inhibiting one target alone does not) occurs with PARP inhibition in tumours with loss of function of *BRCA* genes, or more broadly, homologous recombination deficiency (HRD). Paradigms of this are BRCA-mediated breast and ovarian cancer, but also prostate and pancreas cancer. PARP inhibition and trapping lead to an accumulation of DSB both by preventing repair of SSB and through trapping of PARP at the DNA. DSBs cannot be resolved by cNHEJ or SSB repair mechanisms but can be repaired in cells competent for HR. Tumour-specific inactivating *BRCA* mutations result in deficient HR, and these unrepaired DSBs therefore enable selective cancer cell death [12].

Aberrant regulation of transcription is an important source of endogenous DNA damage in cancer cells. During

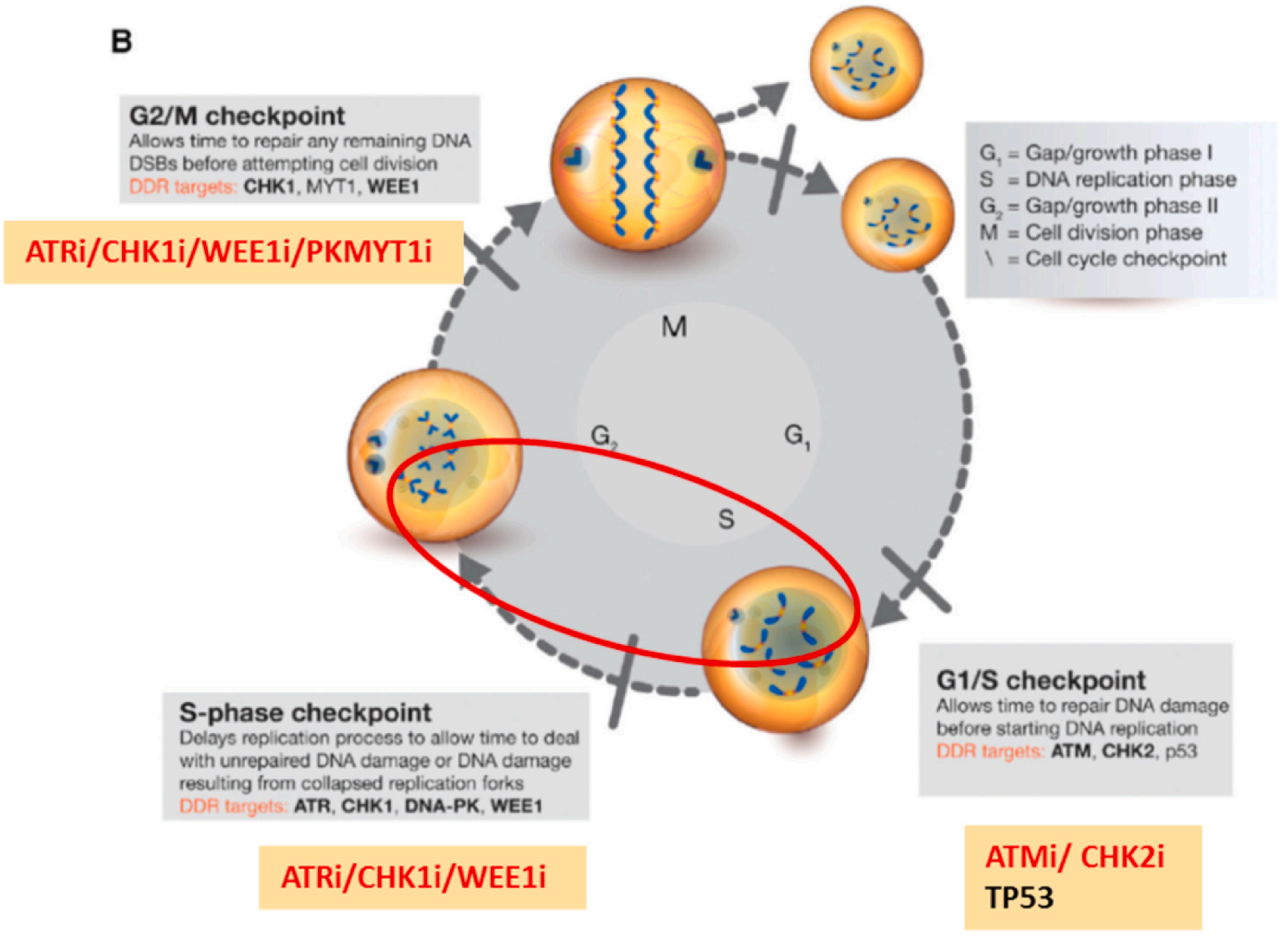

Fig. 2. DNA damage response pathway and the cell cycle.

transcription, the nascent RNA generated by RNA polymerases can hybridise with the DNA template, giving rise to a three-stranded structure called an R-loop [20]. In cancer cells, R-loops can accumulate and interfere with faithful replication, although paradoxically these have also been shown to be involved in mitigating replication stress. In response to aberrant R-loop accumulation, ATR is recruited to facilitate DNA repair. Thus, it has been hypothesised that inhibitors of the ATR-CHK1 pathway may be a particularly effective approach to targeting tumours with R-loop accumulation [23].

Molecular profiling data in paediatric cancers indicate that *BRCA1* and *BRCA2* mutations are very rare (< 2%), as are ATM mutations (< 3%) [24–29]. However, there are alterations in paediatric cancer that could result in synthetic lethality with DDR inhibitors, including ATR inhibitors, for example, *MYC* amplification, aberrant transcription factor gene fusion (*PAX3-FOXO1, EWSR1-FLI1*), increased R-loops, ALT /ATRX mutations and the oncogenic mutator PGBD5 [19,30,31]. For most of these alterations there are preclinical but not clinical data, supporting synthetic lethality. Some examples are

1. *MYC amplification:* In neuroblastoma, combined inhibition of aurora-A kinase and ATR induces rampant tumour-specific apoptosis and tumour regression in *MYCN-amplified* transgenic mouse models [32]. In neuroblastoma cell lines and patient-derived xenograft (PDX) models, ribonucleotide reductase subunit M2 (RRM2)-CHK1 inhibition acts synergistically, illustrating the therapeutic potential [33], and chromosome 11q loss and *MYCN* amplification demonstrate synthetic lethality with CHK1 inhibition, possibly due to inactivation of ATM on 11q [34].
2. *Aberrant transcription factor gene fusion: EWS-FLI1* and *EWS-ERG* translocations have been shown to sensitise Ewing sarcoma cells to ATR inhibitors [35,36]. ATR inhibition also causes increased DNA breakage in *PAX3-FOXO1* fusion-expressing alveolar rhabdomyosarcoma models, suggesting that this tumour entity may also be particularly sensitive to ATR inhibition [37,38]. There are many more potentially susceptible paediatric tumour histologies, for example, other fusion-positive solid tumours, several paediatric brain histologies and subgroups of many others with particular susceptibilities.
3. *Increased R-loops* in certain paediatric tumours, for example, in Ewing sarcoma [39] or embryonal tumours with multilayered rosettes (ETMR) [40], may contribute to their sensitivity to DDR inhibitors, including selective agents

discussed as well as conventional DNA-damaging chemotherapy (e.g. topoisomerase 1 inhibition). Potentially, cells with R-loops may also be sensitive to ATR inhibition as ATR is necessary for R-loop resolution [23].

4. *ALT:* The majority of osteosarcomas utilise alternative lengthening of telomeres (ALT) as a mechanism for telomere maintenance [41–43]. ALT renders cells hypersensitive to ATR inhibition since these cells require ATR for telomere maintenance [44]. Furthermore, as there are several paediatric cancers with *ATRX* mutations (e.g. osteosarcoma, neuroblastoma, medulloblastoma, high-grade glioma) and as ATRX inactivation promotes ALT, ATR inhibition may be a rational therapeutic approach for tumours with ATRX loss [45,46].
5. *PGBD5:* Several paediatric tumours have increased endogenous DNA damage from the oncogenic mutator PGBD5, which is synthetically lethal with ATR inhibition in several paediatric tumours [19,30,31]. Preclinical (*in vitro* and *in vivo*) evidence demonstrated that the ATR inhibitor AZD6738 (ceralasertib) has exceptional *in vitro* selective activity against the majority of PGBD5-expressing preclinical childhood tumour models, including rhabdoid tumour, medulloblastoma, neuroblastoma and Ewing sarcoma [31]. The relative PGBD5 expression levels correlate with sensitivity to ATR inhibition. As the aberrant catalytic activity of PGBD5 is restricted to tumour but not normal cells, this opens the opportunity to exploit synthetic lethality with potentially less normal tissue toxicity.

Secondary resistance to PARP inhibitors occurs in adult cancers by several mechanisms, most frequently restoration of HR through BRCA reversion and also hyper-activation of the ATR/CHK1/WEE1 pathway and stabilisation of replication forks [47]. In paediatric cancer, due to the high-level replicative stress, it is conceivable that in most cases there is the risk of primary resistance to single-agent PARP inhibitors.

In summary, the current DDR inhibitor development is driven by findings from the treatment of adult indications, and although paediatric cancer is different, DDR inhibitors likely have important therapeutic roles. Based on the aspects outlined above, single-agent synthetic lethality with DDR inhibitors is unlikely to be widely applicable [48], and therefore, a sensitisation/combination approach is favoured. Combination therapies which take paediatric tumour biology under consideration and enhance the potential for synthetic lethality may help to overcome 'primary resistance'. Thus far, data supporting predictive biomarkers of clinical response in the paediatric setting are scarce, and complementary biomarker analysis must be included in clinical trials. Biomarker profiles are likely to reflect a constellation of findings rather than single-gene alterations. The most informative trial design will be driven by a clear hypothesis with the intent to further investigate responders and non-responders with detailed retrospective molecular analyses to generate a revised or new hypothesis.

3. Products discussed at the Forum and PIPs

Fifteen medicinal products – olaparib (Lynparza®), talazoparib (Talzenna®), niraparib (Zejula®), AZD5305, AZD9574, RP-2119, AZD6738 (ceralasertib), M1774, RP-3500, BAY-1895344 (elimusertib), ACR-368 (prexasertib), AZD1390, M4076, peposertib and RP-6306 – are discussed (Table 1).

Table 1
DNA damage response pathway inhibitors discussed at the Forum

Product	Target	Paediatric clinical trials (academic)	Paediatric investigation plan (PIP)	Company
Olaparib	PARP	6 (5)	+	AstraZeneca
Talazoparib	PARP	5 (4)	+	Pfizer
Niraparib	PARP, CNS penetrant [136,137]	2(1)	+	GSK
AZD5305	PARP-1 selective	0		AstraZeneca
AZD9574	PARP-1 selective, CNS penetrant	0		AstraZeneca
RP-2119	Polymerase theta (Polθ)	0		Repare Therapeutics
AZD6738 (ceralasertib)	ATR	2 (2)		AstraZeneca
M1774	ATR	0		Merck KGaA
RP-3500 (camonsertib)	ATR	2 (0)		Roche
BAY-1895344 (elimusertib)	ATR	1 (1)		Bayer
ACR-368 (formerly prexasertib)[†]	CHK-1	3 (3)		Acrivon
AZD1390	ATM CNS penetrant	0		AstraZeneca
M4076	ATM	0		Merck KGaA
Peposertib	DNA-PK	0		Merck KGaA
RP-6306	PKMYT1	1 (0)		Repare Therapeutics

PARP inhibitors (veliparib, pamiparib, rucaparib) and Wee1 inhibitors were not presented at the Forum.
[†] Presented by an academic clinician.

Text box 1 Key conclusions of the Paediatric Strategy Forum

- DNA damage response inhibitors have a potentially important therapeutic role in paediatric cancers. Their optimal use, including patient selection and combination strategy, remains unknown.
- There is an imbalance between the number of drugs and mechanisms of action and the limited numbers of patients available to enrol in paediatric early-phase trials, so prioritisation and collaboration are essential.
- *BRCA1/2* mutations occur very infrequently in childhood tumours and are not commonly associated. A different response hypothesis is required to that of *BRCA* mutations with PARP inhibitors.
- Monotherapy trials of PARP inhibitors with the same mechanism of action as previously evaluated are not recommended.
- Combinations of PARP inhibitors with targeted radiotherapy, ATR inhibitors or antibody drug conjugates with topoisomerase I payload require evaluation.
- PARP1-specific inhibitors and PARP inhibitors with very good central nervous system penetration require evaluation.
- As ATR, ATM, DNA-PK, CHK1, Wee1, Polθ and PKMYT1 inhibitors are early in paediatric development, there should be an overall coordinated strategy for their development.
- CHK1 inhibitors have activity in desmoplastic small round cell tumour and have a potential role in other paediatric malignancies, such as neuroblastoma and Ewing sarcoma. Access to CHK1 inhibitors for paediatric clinical trials is a high priority.
- Early engagement of regulators in the clinical development of agents for paediatric cancers is critical.
- By aligning scientific, regulatory and payer requirements from the inception of a clinical trial, the fewest number of patients will need to be enrolled to obtain sufficient evidence for scientific and regulatory purposes.
- Future steps include

  - To define and develop potential relevant biomarkers, a meeting between academia and industry will be held.
  - Academic investigators and industry should work collaboratively to collect and investigate the biology of tumours from patients exposed to PARP inhibitors, to analyse and compare responders to non-responders.
  - A focused meeting on ATR inhibitors.

As of October 2022, there are four published agreed PIPs for PARP inhibitors: olaparib (Lynparza®), talazoparib (Talzenna®), veliparib and niraparib (Zejula®). Three of these PIPs are for combination therapy: talazoparib plus liposomal irinotecan, veliparib plus radiotherapy and temozolomide, niraparib plus dostarlimab. The indications are disease-specific in three PIPs: relapsed/refractory Ewing sarcoma (talazoparib), newly diagnosed high-grade glioma (veliparib); relapsed/refractory neuroblastoma and osteosarcoma, with subsequent upfront evaluation planned in newly diagnosed high-risk patients against current standard-of-care regimens if evaluation in relapsed/refractory disease shows safety and promising efficacy (niraparib). One indication is histology-agnostic: HR-mutated solid tumours (olaparib). There are no PIPs yet for the other DDR pathway inhibitors, but in line with the EU Paediatric Regulation a PIP should be submitted soon according to the timing dictated by the Regulation (Table 2).

Details of completed and ongoing paediatric trials of DDR pathway inhibitors are shown in Table 3. In summary, there are 26 relevant paediatric trials, 16 with PARP inhibitors and 14 with other DDR inhibitors (including four with PARP and another DDR inhibitor), involving 11 products (four PARP inhibitors and seven other DDR inhibitors). Most (22/26) are combination trials, nine with irinotecan and/or temozolomide (two including radiotherapy), five with novel agents (two olaparib and ceralasertib [PARP and ATR], camonsertib and talazoparib [ATR and PARP], niraparib and dostarlimab [PARP and PD-1] and RP-6306 and camonsertib [PKMYT1 and ATR]) and six with other combinations (chemotherapy, radiotherapy).

4. Experience with PARP inhibitors in adults: what have we learned?

In 2005 the synthetic lethality of PARP inhibitors and *BRCA* mutations was demonstrated preclinically [49,50] and the first clinical trial of olaparib commenced [51]. In December 2014 olaparib received its first FDA/EMA approval for advanced *BRCA* mutated ovarian cancer [52]. Now PARP inhibitors are approved in ovarian, breast, prostate and pancreatic cancer in various settings, including treatment, maintenance and adjuvant.

Validated predictive biomarkers are critical for maximising benefit and minimising harm from treatment and need to be integrated into clinical trial design. Best responses to PARP inhibitors are observed with biallelic loss of defined homologous recombination repair (HRR) genes [53], although most studies have not examined this. Tumour biopsies, crucial for DNA sequencing (e.g. targeted, whole-exome or whole-genome sequencing), are often performed but mutations are not always necessary and other mechanisms leading to a functional loss of protein may apply

Table 2
Published paediatric investigation plans (PIPs) agreed for DNA damage response pathway inhibitors

Product	Olaparib (AZ)	Talazoparib (Pfizer)	Veliparib (AbbVie)	Niraparib (GSK)
PIP	Modified PIP 2020 (EMEA-002269-PIP01-17-M01)	PIP 2021 (EMEA-002066-PIP01-20) for Ewing sarcoma [Waiver 2021 (EMEA-002066-PIP02–20) for breast/prostate]	Modified PIP 2020 (EMEA-000499-PIP02-10-M01) [Waivers for breast (2017), and ovarian and lung (2018)]	Modified PIP 2021 (EMEA-002268-PIP02-18-M01) [Waivers for prostate cancer (2018, 2019, 2021)]
MoA	PARP	PARP	PARP	PARP
Condition	Malignant neoplasms (except haematopoietic and lymphoid tissue neoplasms)	Ewing sarcoma	High-grade glioma	Malignant neoplasms (except haematopoietic and lymphoid malignancies)
PIP indication	6 months to 18 years with **homologous recombination repair (HRR)** mutated solid tumours	Talazoparib in combination with liposomal irinotecan (I-IRN) for **relapsed/refractory Ewing sarcoma**	**Newly diagnosed supratentorial high-grade glioma**	**Neuroblastoma** (0–18 years) **Osteosarcoma** (0–18 years)
Waiver	0–6 months	0–1 years	0–3 years	None
Deferral	By 2035	By 2027	By 2027	By 2040
Formulation	Capsule, hard Film-coated tablet Age-appropriate oral solid dosage form	Capsule, hard	Capsule Oral liquid	Film-coated tablet Capsule, hard Age-appropriate oral liquid formulation
Clinical	(1) Open-label safety, PK, PD and preliminary efficacy – **relapsed/refractory solid tumours** (including primary CNS tumours) with HRR deficiency (2) Open-label safety and efficacy in R/R non-CNS solid tumours with HRR mutations (3) Randomised → safety and efficacy in **relapsed/refractory non-CNS solid tumours with HRR mutations**	Active, controlled, two-part trial: Part 1 – recommended phase 2 dose, PK and safety of talazoparib + I-IRN vs. TMZ + I-IRN in R/R solid tumours Expansion cohort patients with HRR and double-strand breaks signalling defects Part 2 - randomised, **talazoparib + I-IRN** vs. I-IRN + TMZ in **relapsed/refractory Ewing**	(1) Open-label, non-controlled, dose-escalating PK, safety and activity of veliparib in combination **with TMZ** in R/R malignant CNS tumours (0–18 years) (2) Double-blind, randomised, placebo-controlled → safety and efficacy of veliparib in combination **with RT + TMZ** (3–18 years) with **HGG**	(1) Open-label, multiple dose, two-part trial → PK, safety, activity, acceptability of **niraparib with dostarlimab**: (1b) R/R solid tumours (except CNS) (6 months to 18 years) (2) R/R osteosarcoma or neuroblastoma (6 months to 18 years) (3) Open-label, randomised, controlled efficacy and safety of niraparib + dostarlimab vs. standard of care in **relapsed/refractory osteosarcoma or neuroblastoma** (6 months to 18 years) (4) Open-label, randomised, controlled → efficacy and safety of niraparib + dostarlimab vs. standard of care in **newly diagnosed HR osteosarcoma or Stage 4 neuroblastoma** (0–18 years)

CNS, central nervous system.

[54]. Copy number/re-arrangement assessment calling is challenging but is essential to demonstrate biallelic loss, ploidy and heterogeneity. Furthermore, the limitation of immunohistochemistry analysis is that the presence of protein does not prove that the protein is also functional.

In metastatic prostate cancer, circulating tumour cells [55,56] and cell-free tumour DNA (ctDNA) [57] are of value, but the low tumour fraction median (10–30%) is a disadvantage, as is the reproducibility, feasibility and the interpretability of complex biomarker data. Tumour fraction is crucial, and a value < 30% makes it very difficult to detect homozygous deletions. Furthermore, the allele frequency matters – if this is 50%, then this may be a germline alteration and a very low

Table 3
Summary of completed and ongoing paediatric trials of DNA damage response pathway inhibitors

Product (company)	Target	Paediatric trials				
		Name – NCT	Phase	Treatment	Indication	Status (study start)
Olaparib (AZ)	PARP	NCT04236414 [138]	1	Monotherapy	Relapsed/refractory solid tumours or primary CNS with an HRR deficiency/gene mutation	Recruiting (2020)
		MATCH NCT03233204 [76]	2	Monotherapy	Relapsed/refractory solid tumours, NHL, HCL with defects in DDR genes	Active, not recruiting (2017)
		ESMART Arm D NCT02813135 [77,139]	1/2	Olaparib + irinotecan	Phase I: irinotecan agent of choice; 2 Phase II expansion cohorts: Relapsed/refractory Ewing sarcoma and HR-deficient tumours	Active, not recruiting (2016)
		NCT01858168 [140]	1	Olaparib + temozolomide	Relapsed/refractory Ewing and RMS	Recruiting (2013)
		NCT04417062* [141]	2	Olaparib + ceralasertib	Relapsed/refractory osteosarcoma	Recruiting (2020)
		ESMART Arm N NCT02813135 [78,139]*	1/2	Olaparib and ceralasertib	HR-deficient tumours or tumours with increased replication/transcription stress 2 expansion cohorts at RP2D: (1) HR defective tumours; (2) increased replication/transcription stress	Recruiting (2020)
		NCT04076579 [142]	2	Olaparib + trabectedin	Advanced/metastatic sarcoma	Recruiting (2020)
Niraparib (GSK)	PARP	SARC025 NCT02044120 [73,143]	1	Niraparib + temozolomide and/or irinotecan	Incurable Ewing	Completed (2014)
		SCOOP NCT04544995 [144]	1	Niraparib + dostarlimab	Relapsed/refractory solid tumours	Recruiting (2020)
Veliparib (AbbVie)	PARP	PBTC-033 NCT01514201 [75,145]	1/2	Veliparib + radiotherapy + temozolomide	Newly diagnosed DIPG	Completed (2012)
		NCT03581292 [146]	2	Veliparib + radiotherapy + temozolomide	Newly diagnosed malignant glioma	Active, not recruiting (2018)
Talazoparib (Pfizer)	PARP	ADVL1411 NCT02116777 [72,147]	1/2	Talazoparib + TMZ	Relapsed/refractory tumours (including Ewing)	Completed (2014)
		BMNIRN NCT02392793 [74,148]	1	Talazoparib + irinotecan +/- temozolomide	Relapsed/refractory solid tumours	Completed (2015)
		NCT04497116 [149]*	1/2	Camonsertib alone or + talazoparib or + gemcitabine	Advanced solid tumours	Recruiting (2020)
		ONITT NCT04901702 [87]	1/2	Onivyde + talazoparib or temozolomide	Relapsed/refractory R solid tumours and Ewing	Recruiting (2021)
		RARE 2 NCT05142241 [150]	2	Talazoparib + temozolomide	Advanced rare cancers	Recruiting (2022)
Ceralasertib (AZ)	ATR	NCT04417062 [151]	2	Olaparib + ceralasertib	Relapsed/refractory osteosarcoma	Recruiting (2020)
		ESMART NCT02813135 [78,139]*	1/2	Olaparib and ceralasertib	HR deficient tumours or tumours with increased replication/transcription stress 2 expansion cohorts at RP2D: (1) HR defective tumours; (2) increased replication/transcription stress	Recruiting (2020)
Elimusertib (Bayer)	ATR	NCT05071209 [152]	1/2	Monotherapy	Relapsed/refractory solid tumours	Recruiting (2021)

(*continued on next page*)

Table 3 (*continued*)

Product (company)	Target	Paediatric trials				
		Name – NCT	Phase	Treatment	Indication	Status (study start)
Camonsertib (RP-3500) (Repare/Genentech)	ATR	NCT04855656 [153]*	1	RP-6306 alone or + camonsertib	Advanced solid tumours	Recruiting (2021)
		NCT04497116 [149]*	1/2	Camonsertib alone or + talazoparib or + gemcitabine	Advanced solid tumours	Recruiting (2020)
Adavosertib (AZ)	Wee1	NCT02095132 [154]	1/2	Adavosertib + irinotecan	Relapsed/refractory solid tumours	Active, not recruiting (2014)
		NCT01922076 [155]	1	Adavosertib + radiotherapy	Newly diagnosed DIPG	Active, not recruiting (2013)
		ESMART Arm C NCT02813135 [16,105]*	1/2	Adavosertib + carboplatin	Relapsed/refractory solid tumours (carboplatin rational agent +/- alterations consistent with presumed WEE1 sensitivity including HR deficiency and TP53 mutations); active no recruiting (2016)	Active no recruiting (2016)
ZN-c3 (Zentalis)	Wee1	NCT04833582 [156]	1/2	ZN-c3 + gemcitabine	Relapsed/refractory osteosarcoma	Recruiting (2021)
Prexasertib (Acrivon)	CHK1/2	ADVL1515 NCT02808650 [101,157]	1	Monotherapy	Relapsed/refractory solid tumours (including CNS)	Completed (2017)
		NCT04095221 [102,158]	1/2	Prexasertib + irinotecan	Desmoplastic SRCT or RMS	Active, not recruiting (2019)
		NCT04023669 [159]	1	Prexasertib + cyclophosphamide or gemcitabine	Relapsed/refractory group 3/4 SHH-medulloblastoma	Active, not recruiting (2019)
LY2880070 (Esperas Pharma)	CHK1/2	NCT05275426 [160]	1/2	LY2880070 and gemcitabine	Ewing Sarcoma or Ewing-like Sarcoma	Recruiting (2022)
RP-6306 (Repare)	PKMYT1	NCT04855656 [153]*	1	RP-6306 alone or + camonsertib	Advanced solid tumours	Recruiting (2021)

CNS, central nervous system; DDR, DNA damage response; DIPG, diffuse intrinsic pontine glioma; HCL, Hodgkin's lymphoma; HR, homologous recombination; HRR, homologous recombination repair; NHL, non-Hodgkin's lymphoma; RMS, rhabdomyosarcoma; RP2D, recommended phase II dose; SRCT, small round cell tumour.

* Clinical trials listed twice in the table as they feature combination therapy of two DDR pathway inhibitors.

percentage (< 10%) is probably sub-clonal and may not be clinically relevant [58,59].

An example of PARP inhibitor development in adults with prostate cancer is instructive. Successive trials in metastatic castration-resistant prostate cancer have defined the role of PARP inhibition. The TOPARP-A trial demonstrated that olaparib resulted in an 88% response rate in patients with DDR biomarkers (*BRCA1/2, ATM*, Fanconi's anaemia genes and *CHEK2*); in contrast, there was only a 6% response in patients who were biomarker negative. The crucial element in this all-comers trial design was that the responders and non-responders had molecular analysis (targeted next-generation sequencing, whole-exome and transcriptome sequencing and PCR for copy number) [54]. The TOPARP-B trial validated the association between the DDR gene aberrations and response to olaparib, with *BRCA2* homozygous deletions having the best response [60,61]. There were also responses in patients with *ATM, CDK12* and *PALB2* mutations. The PROfound trial further refined the role of PARP inhibition, supporting a salvage role in metastatic castration-resistant prostate cancer with defective DDR following disease progression on enzalutamide or abiraterone [62,63].

The experience of developing PARP inhibitors in adults with metastatic castration-resistant prostate cancer has highlighted a number of limitations and challenges. These include genomic heterogeneity, especially in advanced disease, early resistance and lack of longitudinal response markers (RECIST fails to capture early signs of resistance and changes in circulating tumour cells may be more relevant) [56,60,64,65]. Furthermore, biomarker selection for enrichment is still suboptimal; there is a need for assays to identify downstream signatures of HR-deficient pathways and the functional status of HR pathway. Additionally, there are difficulties in obtaining tissue and incorporating complex assays into routine clinical practice. Finally, many drug combinations are limited by overlapping toxicities.

In conclusion, drugs targeting DDR show great potential, but the challenge is how to approach their development in children best. The three key elements are (1) innovative trial design; (2) biomarker selection; and (3) rational combination therapy, which is limited by overlapping toxicity. Patients should be selected to enable lower doses for those with hyper-sensitising mutations, and novel dosing schedules should be employed to minimise the impact on normal tissue. The critical challenges are genomic heterogeneity in advanced disease and resistance.

5. Experience with PARP inhibitors in children

In children, to date PARP inhibitors have been evaluated in Ewing sarcomas, tumours with HRD and diffuse intrinsic pontine glioma. Ewing sarcomas are functionally deficient in DNA repair despite lacking mutations in DNA repair genes [66–68]. There is a strong biological rationale for Ewing sarcoma vulnerability to PARP inhibition as the characteristic *EWS-FLI1* or *EWS-ERG* genomic fusion products interact with the DDR protein and transcriptional co-regulator [67]. *EWS–FLI1* increases transcription to cause R-loops and depletes cells of BRCA1, resulting in deficient HR repair [39]. PARP inhibitor-induced cytotoxicity in Ewing sarcoma cells was 10- to 1000-fold higher after administration of the DNA-damaging agents irinotecan or temozolomide [68]. Furthermore, studies demonstrated that talazoparib potentiated the toxicity of temozolomide up to 85-fold in Ewing sarcoma cell lines [69]. Finally, the combination of talazoparib or olaparib with irinotecan and temozolomide led to a complete and durable response in more than 80% of the tumours in an Ewing sarcoma PDX model [68].

In addition to the treatment of Ewing sarcoma, there is also biological and preclinical rationale for PARP inhibition in neuroblastoma. Specifically, PARP inhibitors may be effective for the treatment of neuroblastoma tumours with loss of ATRX function as this results in impairment of DDR by HR and impaired replication fork progression [45]. Additionally, PARP inhibition may be an effective therapy for neuroblastoma with high levels of replication stress, such as in tumours with 11q loss of heterozygosity (LOH) and those with *MYCN* [70,71].

Unfortunately, the promising preclinical activity of PARP inhibition with irinotecan and/or temozolomide for the treatment of paediatric tumours has not been confirmed clinically to date. In clinical trials evaluating PARP inhibitors plus DNA-damaging cytotoxic chemotherapy for the treatment of Ewing sarcoma, the following results have been achieved: (1) ADVL1411 (COG Phase I evaluating talazoparib plus temozolomide) yielded 0/10 objective responses in Ewing sarcoma (two stable diseases for 8 weeks) [72]; (2) in SARC025 (niraparib plus temozolomide or irinotecan) 1/31 patients with Ewing sarcoma had an objective response (PR). Importantly, the median decrease in tumour PARP activity was 89% [73]. (3) BMNIRN (talazoparib plus irinotecan with or without temozolomide), 6/41 patients (Ewing sarcoma [5] and synovial sarcoma [1]) had an objective response, including 5/14 (31%) patients with Ewing sarcoma [74].

Dose escalation in BMNIRN was limited by gastrointestinal and haematological toxicities, constraining the ability to achieve doses in patients equivalent to those modelled preclinically [68,69,74]. The discrepancy between the preclinical response rate in the Ewing sarcoma PDX model in comparison to those observed clinically may reflect the inability to achieve adequate drug concentrations within the tumour due in part to intermittent dose schedules required to achieve a tolerable regimen [74].

Discouraging results have been observed in the treatment of paediatric non-Ewing sarcoma tumour types: (1) PBTC033: (veliparib plus radiation and temozolomide): no difference in survival compared to historical controls; accrual stopped for futility [75]; and (2) paediatric MATCH, APEC1621H (olaparib as a single agent in patients with relapsed/refractory solid tumours with defects in either *BRCA1, BRCA2, ATM, RAD51C or RAD51D*), had 0/6 objective responses and the study was closed due to slow accrual [76]. Both the lack of response and the poor accrual could be due to a non-relevant responder hypothesis, in this population with a low frequency of these mutations.

However, the preliminary results of ESMART Arm D (irinotecanplus olaparib, phase Ib) indicate 3/24 confirmed PRs (osteosarcoma, neuroblastoma, pineoblastoma) and 1 unconfirmed response (rhabdomyosarcoma) in cohort 1 (HRD) and 1/27 CR and 1 PR in cohort 2 (Ewing sarcoma) with ongoing biomarker correlation [77]. Olaparib is now being combined with an ATR inhibitor in ESMART Arm N with 1/18 confirmed PR (pinealoblastoma) and 1 PR after cycle 9 (neuroblastoma) [78].

The majority of combination regimens with a PARP inhibitor in paediatrics have administered the inhibitor for 5 d [72–75]. In contrast, in ESMART a prolonged course of 10 d is given together with low-dose irinotecan [77,78]; this is more in keeping with the duration used in combination trials, using low doses on intermittent schedules, in adults [79].

Combination trials of PARP inhibitors with other classes of products (ATR [four trials]), PD-(L)1 inhibitors and trabectedin are ongoing (Table 3).

6. Strategies to improve response to PARP inhibitors

The first-generation PARP inhibitors are dual PARP1 and PARP2 inhibitors/trappers, with the primary dose-limiting toxicity being haematological toxicity, especially in combination [80]. Only PARP1 inhibition/trapping is necessary for synthetic lethality in HR-deficient models [80,81] whilst PARP2 is essential for erythropoiesis in preclinical studies [82,83]. Therefore, selective PARP1 inhibitors are expected to have a favourable toxicity profile, may enable a higher drug exposure to the target to achieve greater and durable target inhibition, achieve greater anti-tumour activity and potentially enable broader combination options [84,85]. PARP inhibitors with greater central nervous system (CNS) penetration potentially may have a role in CNS malignancies.

In order to address the narrow therapeutic window of talazoparib plus irinotecan, irinotecan is administered as a liposomal formulation (Onivyde) [86], and is now undergoing evaluation in a phase I/II trial (ONITT) [87]. The hypothesis is that this formulation could increase intra-tumoural SN-38 (the active metabolite of irinotecan) concentrations with decreased systemic concentrations, thereby improving response while decreasing toxicity. In the preclinical setting, Onivyde plus talazoparib led to a complete durable response in an Ewing sarcoma PDX model. Other approaches such as altering the schedule of administration or tailoring to variability in the metabolism of irinotecan are less likely to increase intra-tumoural SN-38.

7. Biomarkers for PARP inhibitors

Adults who lack BRCA1/2 mutations may benefit from PARP inhibitor monotherapy, and not all patients with tumours harbouring BRCA1/2 mutations respond to a PARP inhibitor [15]. Therefore in adults a HRDness phenotype beyond the narrow scope of defects in the BRCA pathway has been proposed. In contrast, there is a subset of tumours that demonstrate 'PARPness' (responsiveness to PARP inhibitors in the absence of HRD) [15]. A number of biomarkers have been proposed in adults [15] (Table 4), with biallelic loss of defined HRR genes being the most predictive [5,53].

Pathogenic mutations in HRR genes (normally biallelic or mutation with LOH) such as *ATR, ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, PALB2, PTEN, RAD50, RAD51B, RAD51C, RAD54L*, *CDK12*, *CHEK1*, *FANCL*, *PPP2R2A* and *RAD51D* are considered classical biomarkers. DNA mutation signatures have been proposed, but these have not been confirmed in the paediatric clinical setting, and the definition/identification of these signatures varies [88–92]. The lack of confirmation of these in the paediatric setting is probably multifactorial, including the very small size of cohorts of patients investigated and lack of responders and non-responders undergoing detailed molecular analysis.

SLFN11 (Schlaffen Family Member 11) is a DNA/RNA helicase with many roles [93]. SLFN11 expression is associated with higher levels of lethal DNA DSB and sensitivity to DDR pathway inhibitors [93–97], including talazoparib, and SLFN11 knockout leads to decreased sensitivity to PARP inhibitors [98]. While SLFN11 expression was detected in approximately 50% of adults with small cell lung cancer [99], SLFN11 is expressed mostly in Ewing sarcoma, desmoplastic small round cell tumour (DSRCT) and osteosarcoma. However, a PDX generated from a patient with high SLFN11 expression treated in the Phase I BMNIRN trial did not respond to irinotecan, temozolomide and PARP inhibition. This suggests that SLFN11 may be necessary but not sufficient for promoting sensitivity to DDR pathway inhibitors.

8. Other DNA damage response pathway inhibitors in paediatric tumours

There are very few published results of paediatric trials of DDR pathway inhibitors (Table 3). There is a

Table 4
Potential biomarkers for PARP inhibitors

Biomarker	Details	Findings in adults
Priority genes: Biallelic loss or mutation with LOH of homologous recombination repair (HRR) genes – *ATR, ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, PALB2, PTEN, RAD50, RAD51B, RAD51C, RAD54L, CDK12, CHEK1, FANCL, PPP2R2A* and *RAD51D BRCA1, BRCA2, ATM, RAD51C, or RAD51D*		
Mutations of other genes: e.g. *TP53, NF1, RB1, CDKN2A, FBXW7, PPP2R1A*		
DNA mutation signatures: HRDetec	Mutational signatures of HRDness have been developed through whole-exome or whole-genome sequencing.	Signature 3 Possible [161]
Genomic instability signatures • HRD test Myriad Genetics myChoice • T5 NGS LOH	Determination of Genomic Instability Score (GIS)-algorithmic measurement of loss of heterozygosity; telomeric allelic Imbalance and large-scale state transitions.	Not inclusive enough in defining molecular signatures of HRD tumours or fail to capture mechanisms of PARP inhibitor sensitivity outside of HRDness [162]
Functional biomarkers RDA51 foci assessment,		
Multigene expression signatures	Potential promise as dynamic biomarkers of HR repair function and PARP inhibitor sensitivity. But derived from comparisons of drug-sensitive versus drug-resistant tumour models because results from drug-sensitivity assays in preclinical models have been shown to be highly variable, with low levels of inter-assay [163].	
Protein expression of PARP1, E-cadherin and/or SLFN11		

biological rationale for combining a PARP inhibitor with an ATR inhibitor as (1) PARP inhibitors create PARP-DNA adducts (PARP trapping) and these DNA adducts stall replication forks and need to be repaired by ATR and (2) catalytic inhibition interferes with the repair of SSB, leading to replication fork damage that requires HR repair [35,80]. The Current Arm N of ESMART is evaluating this precise combination (olaparib + ceralasertib) in a molecularly enriched population [78].

CHK1 is primarily a G2M checkpoint inducer and takes part in HR at stalled replication. Prexasertib (previously LY2606368, now ACR-368) is a novel, second-generation, selective dual inhibitor of CHK1 and 2 which abrogates the DDR checkpoint, allowing cells that have sustained DNA damage to prematurely enter mitosis and undergo mitotic catastrophe due to incompletely replicated chromosomes [100]. The single-agent phase I trial of prexasertib was not biomarker selected, had no pharmacodynamics reported and had no responses [101]. However, prexasertib induces regression in DSRCT PDX models. In a phase I/II study of prexasertib and very low dose irinotecan in DSRCT, a tumour where there is an unmet need for new therapies, there was an objective response rate of 32% at all dose levels including in many patients who had previously received and had progressive disease with irinotecan. However, the study was closed to accrual prior to completion due to the discontinuation of prexasertib supply [102].

Four clinical trials have evaluated WEE1 inhibitors in paediatrics: Phase I/II and Phase I (neuroblastoma, medulloblastoma/CNS embryonal tumours and rhabdomyosarcoma) adavosertib in combination with irinotecan [103,104], adavosertib in combination with carboplatin (ESMART – Arm C) [105,106] and WEE1 inhibitor ZN-c3 in combination with gemcitabine in osteosarcoma [107]. Responses have been observed in neuroblastoma and correlations are made with genomic alterations in the DNA repair in ESMART.

Combination strategies for DNA-damaging agents in adults initially exploited chemotherapy (topoisomerase 1 inhibitors, carboplatin, cisplatin and gemcitabine). Approaches using other DDR inhibitors (e.g. PARP, WEE1 and/or PKMYT1 inhibitors), along with ATR/CHK1 inhibitors and immunotherapy, and radiation therapy, warrant evaluation. Overlapping toxicities have limited the ability to maximise dosing, but perhaps novel formulations such as liposomes and antibody drug conjugates (ADC) and alternative schedules have the potential to overcome this.

9. Potential predictive biomarkers of activity of ATR/CHK1 inhibition

ATR/CHK1 is involved in the repair of replication stress, so biomarkers of response can be classified into

four groups [108]. CHK1 is downstream of ATR, and they are both interlinked but do both have separate functions, so a better understanding of the differences with ongoing research is required to separate biomarkers for response. So far, with current knowledge, a clear separation is not possible [47].

1. Alterations that cause replication stress/or high DNA damage: (i) high expression of (fusion) oncogenes such as *CCNE1, MYC, MYCN, P3F1* and *EWS-FLI1*; (ii) expression of endogenous recombinases such as PGBD5; (iii) mutations of other genes: e.g. *TP53, NF1, RB1, BRCA1/2, CDKN2A, FBXW7* and *PPP2R1A*; and (iv) increased SLFN11 [31,37,108].
2. Signs of active replication stress/DNA damage: (i) DNA fibre assays to quantify replication speed/stalling/instability, activation of ATR, CHK1 (phosphorylation); (ii) R-loops; (iii) RPA phosphorylation; (iv) DNA damage in general (pH2AX, pKAP1) and (iv) extrachromosomal DNA [108–111].
3. Synthetic lethalities: (i) ATM loss and ATR inhibition; (ii) SETD2 mutations or KDM4A amplifications and WEE1 inhibition (due to RRM2 levels); (iii) ARID1A or SMARCA4 mutations and ATR inhibition; and (iv) ATRX [108].
4. Markers causing treatment resistance: (i) low expression of FAM122A and resistance to CHK1 inhibitors; (ii) high *MYT1* expression and WEE1 inhibition; (iii) high RAS-MAPK activity and ATR inhibition; and (iv) high AP1 transcription factor (FOS family) expression and ATR inhibition [37,108].

10. ATM, DNA-PK, Polθ and PKMYT1 inhibitors

ATM inhibitors synergise with complementary DNA-damaging chemotherapy such as topoisomerase and PARP inhibitors, and radiosensitise *in vivo* preclinical tumour models [112,113]. DNA-PK inhibitors have a favourable monotherapy toxicity profile and synergise with radiotherapy, topoisomerase 2 inhibitors and PARP inhibitors in preclinical models [114,115]. The effect of DNA-PK inhibitor and radiotherapy combination on normal tissue must be considered to ensure there is a therapeutic window. To date there are no trials of ATM or DNA-PK inhibitors in children.

Polθ is a multifunctional DNA polymerase required for a-EJ and DSB repair. Polθ becomes essential when HR is defective. It is minimally expressed in normal tissue and knockout animals have no phenotype [116]. The highest frequency of Polθ mutations, anticipated to cause susceptibility to Polθ inhibitors, generally those associated with HR-deficiency (e.g. *BRCA1/2*), occur in paediatric CNS tumours and sarcomas, adult ovarian, breast and gastrointestinal cancers. Polθ inhibitors are expected to be well tolerated and may be important in the repair of DNA damage due to irradiation [117] and chemotherapy [118] and may overcome resistance to PARP inhibitors [119].

CCNE1 [120] amplification is among the most commonly amplified genes in osteosarcoma, and overexpression of Cyclin E has potential prognostic significance. PKMYT1 inhibition is synthetically lethal with *CCNE1*-amplified cancer cells, leading to the dysregulation of the G2/M and G1/S cell cycle checkpoints, respectively [121]. In the absence of these critical checkpoints, the cancer cell accumulates massive replication stress and DNA damage, and ultimately dies. Therefore, PKMYT1 inhibition is an interesting therapeutic approach for CCNE1-amplified cancers, including osteosarcoma. There is one ongoing trial of a PKMYT1 inhibitor in patients aged 12 and older.

11. Discussion

11.1. Patient advocates' perspective

Participating patient advocates maintained that collaboration and strategy development among stakeholders are essential to planning future research on DNA response mechanisms. Past clinical work, too often lacking sufficient collaboration involving all relevant stakeholders, provides important lessons for investigators and companies as they design more innovative therapies. Maintaining simultaneous and parallel research in preclinical and clinical settings can provide mutually beneficial insights. Advocates stressed that it will be necessary to establish new structures to sustain collaborations.

Advocates endorsed a joint industry and academic proposal to re-analyse tumour material from patients exposed to PARP inhibitors and compare responders to non-responders to provide further insights. Innovative therapies will require prioritising among multiple drugs in the same class while focusing on children and adolescents most in need.

Advocates also stressed that their involvement in cancer drug development programs, from the earliest stages through clinical trial implementation, can be of substantial benefit to academic and industry investigators. Examples include insights about assessments of patients' quality of life, toxicity reduction strategies, maximal use of tumour material and novel approaches to trial design.

Achieving a long-term benefit for children must be the overarching objective and pervasive aim for any integrated research program. However, while children and adolescents need access to high-quality clinical trials, it is also essential to ensure they have access to these novel agents once safety and early signals of efficacy are established.

11.2. General themes

The DDR pathway is a relevant pathway in paediatric malignancies despite the low frequency of *BRCA1* and *BRCA2* (< 2%) and *ATM* mutations (< 3%) [24–29], and

the different molecular and immune landscape of children's tumours compared to adults. Despite this, DDR inhibitors have been explored less in paediatrics compared to other classes of agents (chemotherapy, targeted inhibitors and immunotherapy). The response hypothesis initially investigated for paediatric tumours relating to *BRCA1* and Ewing sarcoma may not be relevant; therefore, new (different from adults) response hypotheses for replication stress in children have to be generated. There is a need for more consistent preclinical evaluation with panels of representative models [122] and 'distribution/sharing' of access to new models, including both front-line, naive-therapy models and relapsed, multitreated models. An emphasis on collaboration by sharing preclinical models is paramount to the success of new clinical trials that maximise clinical benefit in patients. A further question is whether patient selection has been appropriate with the current lack of biomarkers to select patients.

Collaboration is mandatory at the pre- and clinical levels; there should be even greater interaction between the European Innovative Therapies for Children with Cancer (ITCC) P4 project and the North American PIVOT project [123,124], two large platforms that have established and molecularly characterised large series of PDX models for many different types of paediatric cancers. Both platforms facilitate a rapid *in vivo* preclinical testing of new drugs and rational combinations, and the identification of putative response biomarkers, resulting in a better prioritisation of drugs and patients selected for clinical trials. These preclinical platforms provide the hypothesis for selection biomarkers, which then can be evaluated in clinical trials. Biological studies should be carried out retrospectively, but critically they should be incorporated as key components in new clinical trials based on preclinical derived responder hypotheses. Biomarker studies should be maximised, and this highlights the importance of tissue availability and pharmacodynamic readouts, as well as evaluating the value of circulating DNA. Clinical trials should be executed through the rigorously established paediatric oncology early trial networks, and there are major advantages of trans-Atlantic platform trials, particularly with the focus on rare genomically defined subgroups.

A combination of a PARP, ATR, CHK1 or other DDR inhibitor with an ADC linked to a topoisomerase I inhibitor payload may be of particular benefit by focusing the topoisomerase I inhibitory activity on cancer cells and away from normal tissues, thereby creating the potential for enhanced ADC activity that is tumour selective.

11.3. Regulatory considerations

Early engagement of regulators in the clinical development of agents for paediatric cancers is critical. Trial design needs to consider regulatory requirements (PIP and iPSP) along with a full clinical development pathway, including early- and late-phase combination trials for novel agents, and for all drugs included in the combination (depending on emerging results from the early-phase studies, i.e. an iterative process). By aligning scientific, regulatory and payer (e.g., European health technology assessment bodies) requirements from the inception of a clinical trial, the lowest scientifically justified number of patients needs to be enrolled to obtain sufficient evidence for scientific and regulatory purposes [3]. Furthermore, there should be simultaneous regulatory submissions of individual PIPs and iPSPs to the EMA and FDA, respectively, in order to facilitate early regulatory interactions, for example, at Paediatric Regulatory Cluster Calls in view of a global development [125–128]. An agreed PIP is a living document which can be modified and evolved in light of new evidence – the stepwise PIP. The main goal is to foster evidence generation to inform life cycle PIP considerations and support developments based on needs and robust science [129].

11.3.1. Paediatric formulation

The development of oral, 'child-friendly' formulations, including palatable suspensions, liquid formulations or oral dispersible tablets or mini tablets of the medicinal products that are appropriate to be administered to young children, is critical. Some of these may be administered by gastric tube for patients unable to take oral medication. While expensive to create, this process is essential to making available the most promising compounds to patients of all ages.

11.4. Specific themes

11.4.1. What are the lessons learnt from the development of PARP inhibitors?

PARP inhibitors have been evaluated in paediatrics for over 10 years and a rational biological hypothesis for Ewing sarcoma (from preclinical to clinical) has been explored and does not appear to be valid, particularly for single-agent PARP inhibitors and for combinations of PARP inhibitors with temozolomide. Experience of PARP inhibitors with temozolomide provides a cautionary note for combinations of cytotoxic agents with DDR pathway inhibitors: the strong potentiation of temozolomide by PARP inhibitors observed in preclinical models (*in vitro* and *in vivo*) could not be translated to clinical success due to the combinations' potentiation of toxicity in normal tissues [68,69,72,74]. Initial results for the combination of a PARP inhibitor plus a topoisomerase I inhibitor were also disappointing, but final conclusions await the results of the ONITT phase I/II trial [87]. There have been very few clinical responses to PARP inhibitors in children. Currently, there are four PIPs for PARP inhibitors (olaparib, talazoparib, veliparib, niraparib), three for combination therapy

and only olaparib for monotherapy. There are no validated biomarkers to identify them.

11.4.2. Future directions for PARP inhibitors

Future evaluation of PARP and DDR should include assessment of novel drug combinations, informative trial designs driven by a clear hypothesis with the intent to further investigate responders and non-responders with detailed retrospective molecular analyses to generate a revised or new hypothesis and collaboration to determine the 'best' biomarkers. As with other combinations, the therapeutic-to-toxicity window of regimens evaluating PARP inhibitors and other agents may be limiting. Additional dosing strategies that minimise the cytotoxic agent and maximise the DDR inhibitor should also be explored. The current lack of biomarkers to select patients may account for the lack of clinical activity despite promising preclinical results. Further investigation of the role of both HRR gene mutations and genomic instability, determined by assessing genomic scarring or gene signatures, is needed to inform the selection of paediatric patients for clinical trials. There is no rationale to enrol children in trials of monotherapy with PARP inhibitors with the same mechanism of action as those already tested. A PARP1-specific inhibitor requires evaluation to determine whether this class of product has advantages (with a very short monotherapy phase); similarly, PARP inhibitors with very good CNS penetration, such as AZD9574 [130], require assessment. Combinations of the current PARP inhibitors with chemotherapy (except topoisomerase inhibitors) are highly unlikely to be effective, in view of toxicity constraints. Combinations, especially of novel-novel agents, need to be explored with a strong biological rationale and preclinical evidence; for example, MIBG therapy for patients with *ATRX* deletions, ATR inhibitors, Polθ inhibitors, radiotherapy, topoisomerase inhibition (not as chemotherapy, but as novel formulations, e.g. ADC) or immunotherapy. The majority of paediatric tumours are immunologically cold; therefore, activity combining anti-PD-(L)1 and PARP inhibitors is required from ongoing randomised phase III trials in adult patients with immunologically cold tumours as variable activity has been reported from phase I/II trials [131–134].

11.4.3. CHK-1 inhibitors

This is an important class of products in paediatrics because of (1) their activity in DSRCT (tumours which are resistant to many other therapeutic approaches); (2) preclinical research demonstrating their activity in neuroblastoma and (3) their crucial role in the DDR pathway. Availability of CHK-1 inhibitors, in particular ACR-368 (prexasertib), for clinical trials in DSCRT, neuroblastoma and testing strong biological hypotheses, is a very high priority.

11.5. ATR inhibitors

In view of their applicability to paediatric tumours, ATR inhibitors are a very interesting class of products, relatively early in development in paediatrics, and none of the four ATR inhibitors in clinical development have been approved in adults yet. An integrated and coordinated strategy for their paediatric development would benefit children, industry and clinicians.

11.6. Approach to prioritising DNA damage response pathway inhibitors

It was concluded, based on the experience with PARP inhibitors, that the following rational approach to prioritise DDR pathway inhibitors in the future should be adopted:

1. Evidence for mechanism-based tumour-regressing single-agent activity: This evidence can come from clinical trials in adults or it can derive from paediatric *in vivo* preclinical studies using well-credentialed models. Beyond some proposed synthetic lethal interactions (and by analogy to PARP inhibitors and *BRCA1/2* mutations), single-agent activity is dispensable. For example, ATR (and even more so ATM and DNA-PK inhibitors) have very limited monotherapy activity in adults.
2. If there is no single-agent activity and clinical efficacy is only expected through the use of combination, then one or both of the following (ideally both) are important:
   a. Proof-of-concept from adult cancers: There is replication stress in adult cancers and having strong evidence that a proposed combination of a DDR pathway inhibitor plus another anti-cancer agent has a therapeutic window for one or more adult cancers is important. When preclinical data for a combination is strong in adult cancer preclinical models, then the inability to establish a therapeutic window in adults likely reflects dose-limiting adverse effects of the combination on normal tissues. This is a red flag as it suggests that there is not adequate separation between the effects of the combination on replication stress in cancer cells versus the combination's effect on normal tissues.
   b. Preclinical *in vivo* data are generated by panels of representative paediatric cancer models, for example, in the context of the EU-TANSCAN funded BRCAddict project [135] to better address the tumour heterogeneity in children, showing evidence for combination activity in which the addition of the DDR pathway inhibitor markedly potentiates the activity of the agent to which it is added. It is essential to confirm that the drug levels in the preclinical setting approximate the drug levels achievable in the clinic.
3. A way to select patients who are likely to respond based on either diagnosis or a biomarker.

11.7. How to best characterise biomarkers to identify the optimal patient populations?

There are many potential biomarkers proposed. An integrated strategy and a consensus, both in academic and industry trials, of the investigations and biomarkers to be explored would greatly enhance efficiency. Tumour biopsies, prior to therapy, with a common portfolio of investigations for DNA sequencing are crucial prior to therapy in order that the responders and non-responders have molecular analysis, helping to validate responder hypotheses; however, the ethical aspects require discussion with patient advocates. Response biomarkers (including ctDNA) should be incorporated to detect treatment failure/emerging resistance early. ctDNA is of potential value but can have disadvantages as detection of allelic imbalance is challenging with low tumour fraction in cell-free DNA. However, the detection of a point mutation is feasible even with a very low fraction of ctDNA. In addition if a very low allelic fraction of a point mutation is detected, the interpretation of allelic fraction is complicated when the actual tumour fraction is not known which is common in circulating tumour DNA analysis. No circulating tumour cell assays have been validated in paediatric disease. In the design of novel-novel combinations, data generation of pharmacokinetics and safety of each of the components, including information on the individual contribution of the combinational partner(s), should be considered.

11.8. What are the best trial designs to evaluate these agents?

Molecular- and hypothesis-driven proof-of-concept trials are crucial. The most informative trial design will be driven by a clear hypothesis with the intent to further investigate responders and non-responders with detailed retrospective molecular analyses to generate a revised or new hypothesis.

An innovative trial design, including platform trials, should be employed. Approaches include 'pick the winner' designs and adaptive (Bayesian) models. Flexible dosing regimens of novel combination should be employed.

11.9. What are rational combinations, based on the knowledge of biology, which may be novel-novel combinations?

Combinations should be based on and test robust biological hypotheses, underpinned by preclinical *in vivo* data generated by panels of representative models and/or adult clinical studies. ITCC P4 [123] and PIVOT [124] are well placed to provide these preclinical data. The principles employed in prioritising single agent for evaluation, as described above, should be employed. Novel-novel combinations of agents with non-overlapping toxicity should be evaluated, and there should be caution of combinations with chemotherapy as overlapping, particularly haematological, toxicity may limit the ability to deliver clinically active doses. There is a strong rationale for combinations of PARP and ATR inhibitors.

12. Conclusion

The DDR pathway is important in cancer drug development in children, but strategies must be cognisant of the different biology in childhood tumours compared to adults (Text Box 1). Rational combinations and approaches need to be designed based on the relevant biology. The overall approach should be collaboration and prioritisation. Investigators should learn the lessons from the past and apply them to the future, working collaboratively – research efforts should be linked, integrated and sustainable. The most informative trial design will be driven by a clear hypothesis with the intent to further investigate responders and non-responders with detailed retrospective molecular analyses to generate a revised or new hypothesis.

There has been a substantial delay in the paediatric development of DDR pathway inhibitors. Four years elapsed between the first trial in adults of a PARP inhibitor and the first-in-child trial, and four years between the first regulatory approval of olaparib in adults by the EMA and FDA and approval of a PIP for olaparib. In the future, for the benefit of children, this timeline should be shorter.

Three major concrete actions will be taken as a result of this Forum: (1) a consensus between academia and industry on the relevant biomarkers will be developed in early 2023; (2) academic investigators and industry to work collaboratively to collect and investigate the biology of tumours from patients exposed to PARP inhibitors, to analyse and compare responders to non-responders to provide further insights; and (3) a focused meeting on ATR inhibitors in mid-2023 will be convened. As the paediatric development of the four ATR inhibitors currently in development is in its early phase in paediatrics, with no drugs yet approved in adults, coordination of their development through early interactions with regulators, ensuring timely and adequate data generation to support subsequent PIPs life cycle decision-making based on evidence, with the same type of biomarker information generated in each paediatric trial and commitment from the pharmaceutical industry to merge the clinical/biological data to inform strategic decisions, would be enormously advantageous.

In order that inhibition of the DDR pathways in children with cancer is maximally explored and if determined beneficial, development should be based on the relevant biology, simultaneous and parallel research in preclinical and clinical settings, and an overall collaborative strategy, including all stakeholders.

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Declaration of Competing Interest

IG is an employee of Merck Serono Ltd, Feltham, UK, an affiliate of Merck KGaA, Darmstadt, Germany. TJU is an employee of Repare Therapeutics, Cambridge, MA, USA. TJH is an employee of GSK, Collegeville, PA, USA. BRD is an employee of AstraZeneca, Cambridge, UK. JC is an employee of Pfizer, Tadworth, UK. RB is an employee of Genentech, a Member of the Roche Group, South San Francisco, CA, USA. ADJP has consulted for Lilly, Norgine and Developmental Therapeutics Consortium Limited and been an advisor for Amgen. All remaining authors have declared no conflicts of interest.

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Appendix

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